

## Chronicity of infection with *Treponema paraluis-cuniculi* in New Zealand white rabbits

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**SUMMARY** Popliteal lymph nodes from eight New Zealand white rabbits with clinical or serological evidence of naturally acquired infection with *Treponema paraluis-cuniculi* were transferred to rabbits that had not been exposed to this infection. Lymph nodes from two rabbits successfully transmitted infection. The nodes from one of these rabbits transmitted infection during both the acute and chronic stages of infection. Recipients that were successfully infected showed concomitant antibody responses in the Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and fluorescent treponemal antibody-absorption (FTA-ABS) tests six to 10 weeks after inoculation; recipients of uninfected nodes showed no change in serological state. Antibody responses were followed by the development of dark field positive genital lesions 14 to 15 weeks after inoculation.

### Introduction

*Treponema paraluis-cuniculi* is the aetiological agent of venereal spirochaetosis in rabbits. The organism is antigenically related to *Treponema pallidum*, the cause of syphilis in man. This antigenic similarity allows the use of serological tests for human syphilis to diagnose venereal spirochaetosis in rabbits. The reported prevalence of non-specific reagin and specific treponemal antibodies in various rabbit populations differs.<sup>1-5</sup> In particular, the Venereal Disease Research Laboratory (VDRL) test gives a higher percentage of reactors than either the fluorescent treponemal antibody absorption (FTA-ABS) or the *T pallidum* immobilisation (TPI) tests. Although rabbits had no evidence of venereal spirochaetosis when they were evaluated, most studies have not excluded the possibility of latent infection with *T paraluis-cuniculi*.<sup>4,6,7</sup> This study sought to discover whether *T paraluis-cuniculi* persists after remission of clinical venereal spirochaetosis in naturally infected rabbits. Antibody responses in rabbits were compared using various serological tests after experimental infection by transfer of lymph nodes. The cell mediated immune response to infection with *T*

*paraluis-cuniculi* was examined in naturally and experimentally infected rabbits.

### Materials and methods

#### ANIMALS

All rabbits were New Zealand whites obtained from a commercial vendor. Rabbits were caged individually, maintained at 18-24°C, and given antibiotic free feed and water. We studied 11 rabbits with clinical or serological evidence, or both, of venereal spirochaetosis. Two (1D and 2D) were diagnosed in the university's animal facility and the remainder were from the vendor's breeding colony.

#### CLINICAL SPECIMENS

Suspect cutaneous lesions were abraded with sterile gauze soaked with 0.9% saline. Serum from lesions was expressed on to glass slides. Smears for dark field microscopy were mounted with a coverslip, placed in a moisturised chamber, and examined immediately. Smears for fluorescent antibody dark field examination were air dried, fixed, and stained as described elsewhere.<sup>8</sup> *T pallidum*, Nichols strain, was used as a positive control.

#### SEROLOGICAL TESTS

Blood was collected from the marginal ear vein, allowed to clot in vacutainer tubes without anti-coagulant, centrifuged, and the serum removed.

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Serological tests were performed as described in the *Manual of tests for syphilis*.<sup>9</sup> A quantitative VDRL slide test on serum was performed using commercially obtained reagent (Difco Laboratories, Detroit, Michigan, USA). The rapid plasma reagin (RPR) card test was performed on serum using a commercial kit (Kit No 110, Hynson, Wescott & Dunning, Baltimore, Maryland, USA). Bactosyphilitic serum 4+ (Difco) was used as the positive (reactive) control. In general, the FTA-ABS test was performed as described. Serum samples were diluted 1/5 in sorbent; further dilutions were made in phosphate buffered saline (PBS). *T pallidum* (Nichols strain) antigen smears were covered with 0.03 ml test serum dilutions. After the smears had been rinsed, goat anti-rabbit IgG (1/1600) conjugated with fluorescein isothiocyanate (Cappel Laboratories, Cochranville, Pennsylvania, USA) was added. Reactive control serum samples were obtained from rabbits that had been experimentally infected with *T pallidum* or *T paraluis-cuniculi*. Negative control serum samples were obtained from rabbits that gave negative reactions to the RPR and VDRL tests (normal rabbit serum). Results were presented as the highest serum dilution giving a 2+ degree of fluorescence.<sup>10</sup>

#### TRANSFER OF LYMPH NODES

We removed popliteal lymph nodes from donor rabbits by aseptic operation as described previously.<sup>11</sup> Rabbits were anaesthetised with 4% halothane and masked on an open system using a Dupaco anaesthesia machine. All rabbits made satisfactory recoveries.

The popliteal lymph nodes were placed in a sterile petri dish; fat and connective tissue were removed. Transverse sections of the nodes were dispensed into: (a) cold solution of 1% glacial acetic acid in 95% ethanol for tissue immunofluorescence; (b) Eagle's minimum essential medium with 10% normal rabbit serum, inactivated at 56°C for 30 minutes, for use in lymphocyte transformation tests; and (c) 10% neutral buffered formalin for histopathology. Lymph expressed from the nodes was imprinted on glass slides and examined for the presence of treponemes by dark field and fluorescent antibody dark field microscopy. A volume of 1 ml of 50% normal rabbit serum in 0.9% saline, inactivated at 56°C for 30 minutes, was added to the remaining lymph node fragments. These fragments were minced with sterile scissors, and 0.5 ml of the fluid was drawn up into a syringe for inoculation into recipient rabbits.

Extracts from popliteal lymph nodes from eight donors (D) were transferred to 10 recipients (R). In two rabbits (7D and 8D) lymph nodes were transferred at intervals of five months. Rabbits used as lymph node recipients were virgin young adult males

(3.0 to 4.5 kg) from the same rabbitry, that had no clinical signs of venereal spirochaetosis and no serum antibodies to *T paraluis-cuniculi* in the RPR and FTA-ABS tests. Recipient rabbits were sedated with 40 mg/kg ketamine hydrochloride intramuscularly. The width of the testes were measured, the scrotum swabbed with 70% ethanol, and medium from minced lymph nodes of donors inoculated into the left testicle. A similar amount of 50% inactivated normal rabbit serum in 0.9% saline was inoculated into the right testicle (control). Rabbits were examined weekly for signs of venereal spirochaetosis, and the testes were measured. Blood samples were obtained from an ear vein every two weeks for serological tests. Recipient rabbits were followed up for a minimum of 14 weeks after inoculation. At the end of the observation period rabbits were sedated with 50 mg/kg ketamine hydrochloride given intramuscularly, and 25 ml blood was collected in a heparinised syringe by intracardiac puncture. Rabbits were killed in a carbon dioxide chamber, and sections of spleen and popliteal lymph nodes were placed in Eagle's minimum essential medium with 10% inactivated normal rabbit serum for lymphocyte transformation tests. The testes were removed and placed in 10% neutral buffered formalin for histopathology. Similarly, at variable times after removal of lymph nodes donor rabbits were sedated, bled by intracardiac puncture, and killed. Sections of spleen and popliteal lymph nodes were collected from them and processed as from recipient rabbits.

#### LYMPHOCYTE TRANSFORMATION

Lymphocytes were obtained from heparinised blood by Ficoll-Hypaque separation. Spleens and popliteal lymph nodes were removed aseptically and placed in cold Eagle's minimum essential medium with 10% inactivated normal rabbit serum. Lymphocyte suspensions were obtained from spleens and lymph nodes by gentle teasing. All lymphocytes were washed in balanced salt solution and placed in micro-culture as described previously.<sup>12</sup>

Sonicated *T pallidum* (Nichols strain) prepared as described previously,<sup>12</sup> was used as the treponemal antigen at a concentration of  $50 \times 10^6$ /ml. Concanavalin A (con A), a rabbit T cell mitogen, was obtained commercially (Miles Laboratories, Elkhart, Indiana, USA) and used at a concentration of 5-10 µg/ml. Cultures were pulsed with 0.1 µCi <sup>125</sup>I-iododeoxyuridine (IUdR) for 24 hours and the incorporated counts per minute (cpm)/ $0.25 \times 10^6$  lymphocytes were counted. The geometric mean of six replicate cultures for each sample was calculated; the background values for unstimulated cultures (about 50 to 450 cpm) were subtracted. Values obtained for different rabbits were compared with

those for uninfected rabbits using Student's *t* test, with  $p < 0.05$  defined as being significant. The stimulation index was derived from dividing the mean value for cultures stimulated with *T pallidum* or con A by the mean for unstimulated cultures. The mean stimulation index of splenic lymphocytes from five uninfected rabbits was 1.5 (range 1 to 1.8) for *T pallidum* and 92.9 (range 61.5 to 147) for con A.

#### TISSUE IMMUNOFLUORESCENCE

The presence of treponemes in popliteal lymph node sections was investigated by a fluorescent antibody staining technique. Lymph node samples were removed aseptically and placed immediately in cold 1% glacial acetic acid in 95% ethanol, and sections were prepared as described previously.<sup>13</sup> A double antibody method was used to label serial sections of the tissue samples. Sections were flooded with the appropriate dilution of human antiserum to *T pallidum* followed by rabbit anti-human IgG conjugated with fluorescein isothiocyanate (Beckman Diagnostics, Fullerton, California, USA). Controls included tissues stained with normal human serum as the first antibody and uninfected tissues stained as described. Tissue sections were examined with a Zeiss fluorescent microscope with Phloem illumination.

#### HISTOPATHOLOGY

Tissues were fixed in 10% formalin buffered with 2% sodium acetate, cut into 6  $\mu$ m sections, and stained

with haematoxylin and eosin. Selected tissues were stained to show spirochetes by the Warthin-Starry silver impregnation technique.<sup>14</sup> Histopathological findings in the popliteal lymph node were graded as being 0 (absent), 1 (mild), 2 (mild to moderate), 3 (moderate), 4 (moderate to severe), or 5 (severe) by one of us (WEG) who did not know the rabbits' clinical state. Features graded were nodal enlargement, lymphoid hyperplasia, neutrophilic infiltration, histiocytic hyperplasia, and capsular fibrosis. An inflammation score, which was the sum of the foregoing, was calculated to compare the severity of lymphadenitis. The inflammation scores of the popliteal nodes of five uninfected rabbits ranged from 4 to 8.

#### Results

##### NATURAL INFECTION (DONORS)

Various aspects of naturally acquired infection with *T paraluis-cuniculi* were examined in 11 rabbits, ranging in age from 11 to 35 months (the ages of two were unknown) (table 1). Six were males and five were females. Rabbit 8D-1 had clinical evidence of venereal spirochaetosis with lesions that were positive on dark field and fluorescent antibody dark field microscopy. Rabbit 1D had had clinical evidence of venereal spirochaetosis 35 weeks previously, which had been treated at its onset with procaine penicillin G 50 000 units/kg once daily for seven days; the lesions had regressed within eight days. Infection in

TABLE 1 Characteristics of naturally acquired infection with *Treponema paraluis-cuniculi* in New Zealand white rabbits at time of lymph node transfer

No of rabbit	Age (months)	Sex	Evidence of infection	Titre of serological reaction in:			Treponemes in transferred popliteal lymph nodes shown by:				
				VDRL	RPR	FTA-ABS	DF	FADF	FA	SI	IS
Donors:											
1D	Adult	F	Clinical signs (treated) 35 weeks before	—	—	1/16	—	—	NT	NT	6
2D	Adult	M	Seropositive for > 5 months	—	—	1/4	NT	—	NT	1.0	7
3D	11	M	Seropositive for > 3 months	1/1	1/2	1/128	NT	—	NT	4.5*	8
4D	22	M	Seropositive	—	1/1	1/128	—	—	NT	NT	23
5D	19	F	Seropositive	1/2	1/1	1/8	—	—	NT	8.7*	8
6D	18	F	Seropositive	1/8	1/8	1/256	NT	—	NT	2.1*	8
7D-1	26	F	Seropositive	1/4	1/4	1/512	—	—	—	15.1*	7
8D-1	17	F	Active clinical signs for 1 week	1/2	1/1	1/16	—	—	—	1.2	17
7D-2	31	F	Seropositive for > 5 months	1/16	1/4	1/16	NT	NT	NT	3.4*	NT
8D-2	22	F	Clinical signs (untreated) 23 weeks before	1/8	1/8	1/128	NT	NT	NT	1.0	NT
Non-donors:											
9	31	M	Seropositive for > 7 months	1/1	1/2	1/16	—	—	—	1.0	9
10	35	M	Seropositive for > 6 months	1/2	1/1	1/64	—	—	—	1.3	11
11	24	M	Seropositive for > 5 months	1/4	1/2	1/64	—	—	—	22.2*	14

VDRL = Venereal Disease Research Laboratory test; RPR = rapid plasma reagin test; FTA-ABS = fluorescent treponemal antibody absorbed test.

DF = dark field microscopy; FADF = fluorescent antibody dark field microscopy of lymph; FA = fluorescent antibody staining of lymph nodes.

SI = Stimulation index (mean counts per minute <sup>125</sup>IUdR incorporated by cultures stimulated with *T pallidum* or concauavalin A divided by mean for unstimulated cultures.) \* =  $p < 0.05$ .

IS = Inflammation score (severity of lymph node lesions.)

NT = Not tested.

— = Negative test result.

TABLE II Response to *Treponema pallidum* and concanavalin A (con A) of lymphocytes from various tissues of New Zealand white rabbits with naturally acquired infection with *Treponema paraluis-cuniculi*

No of rabbit	Weeks after transfer of lymph node	Response of lymphocytes from spleen to:				Response of lymphocytes from blood to:				Response of lymphocytes from popliteal lymph node to:			
		T pallidum		Con A		T pallidum		Con A		T pallidum		Con A	
		Cpm	SI	Cpm	SI	Cpm	SI	Cpm	SI	Cpm	SI	Cpm	SI
Donors:													
1D	21	743*	3.2	13 144	94.2	240*	2.2	26 402	130.4	NT	NT		
2D	15	24	1.2	1 841	14.8		NT			NT	NT		
3D	19	917*	7.3	29 411	202.4	791*	5.3	17 048	93.7	NT	NT		
4D	8	106*	1.6	11 007	66.5		NT			NT	NT		
5D	19	748*	3.6	30 110	103.8	39	1.1	4 355	24.0	281*	3.4	478	7.2
7D	0†	389*	7.1	845	8.2	14	1.1	38	1.2	0	1.0	2	1.0
8D	0†	0	1.0	709	8.0	34	1.1	119	2.2				
Non-donors:													
9	NT	76	1.3	42 508	219.1	110*	2.0	17 883	160.7	0	1.0	36 945	148.8
10	NT	65	1.3	21 615	107.0	23	1.1	18 873	115.4	29	1.3	25 303	198.7
11	NT	630*	3.7	5 759	23.2	115*	1.8	22 484	240.2	1 930*	22.2	12 663	71.7

Cpm = Mean counts per minute  $^{125}$ IUdR incorporated. \* =  $p < 0.05$ .

SI = Stimulation index (for explanation see table I).

† = Weeks after second lymph node transfer. NT = Not tested.

other rabbits was detected by various serological tests. Hence one rabbit (8D-1) was thought to be in the acute stage of infection, while all others except the treated rabbit were considered to be in the chronic phase because of the absence of clinical signs of venereal spirochaetosis and the presence of serum antibodies.

To assess the persistence of naturally acquired infection with *T. paraluiscuniculi* in rabbits, the popliteal lymph nodes were examined in detail (rabbits 7D and 8D were evaluated twice at intervals of five months (table I)). Dark field and fluorescent antibody dark field examination of fluid expressed from lymph nodes and fluorescent antibody staining of tissue sections of lymph nodes showed no evidence of treponemes. Five of nine rabbits tested had a significantly greater uptake of  $^{125}\text{IUdR}$  by lymphocytes from lymph nodes stimulated by sonicated *T. pallidum* than by unstimulated control cells. The stimulation indexes ranged from 2.1 to 22.2. Histopathological examination showed that all lymph nodes had varying degrees of enlargement due primarily to lymphoid hyperplasia. Neutrophil infiltration, histiocytic hyperplasia, and capsular fibrosis were also present. The severity of most of the lymph node lesions, summarised in a total inflammation score, ranged from 6 to 9, although four had scores between 11 and 23. Rabbit 8D, which had acute infection, had an inflammation score of 17; the onset of infection in other rabbits with high inflammation scores could not be assessed. There was no relation between the inflammation scores and the lymphocyte stimulation indexes.

The uptake of  $^{125}\text{IUdR}$  and the stimulation indexes of lymphocytes from various tissues of rabbits with naturally acquired *T. paraluiscuniculi* infection were also measured at necropsy (table II). In six of ten rabbits, the uptake of  $^{125}\text{IUdR}$  by splenic

lymphocytes exposed to *T. pallidum* was significantly greater than in the control. This also occurred in four of eight specimens of blood lymphocytes and two of five popliteal lymph node specimens. Three rabbits had no significant lymphocyte responses from any of the three tissues. However, the stimulation index for con A in two of these rabbits was also low. The responses of lymph node and splenic lymphocytes to *T. pallidum* antigens correlated well in the rabbits examined, whereas peripheral blood lymphocytes did not.

#### EXPERIMENTAL INFECTION (RECIPIENTS)

To delineate further the persistence of *T. paraluiscuniculi* in lymph nodes of naturally infected rabbits and to assess the serological response after experimental infection, fluid from the popliteal lymph nodes of each of eight naturally infected rabbits was inoculated into eight normal rabbits. Single popliteal nodes from rabbits 7D and 8D were removed and inoculated at intervals of five months, giving a total of 10 experimentally inoculated rabbits.

Three rabbits (6R, 8R-1, and 8R-2) developed genital lesions that were positive on dark field and fluorescent antibody dark field microscopy. They seroconverted with material from rabbits 6D and 8D (table III). Rabbit 6D was a seropositive breeding female, with no clinical signs of venereal spirochaetosis. Rabbit 8D had clinical evidence of venereal spirochaetosis for 10 weeks; the first transfer was made one week after onset of lesions and the second 13 weeks after remission of lesions. Seroconversion was the first indication that the inoculation was successful. The FTA-ABS test was usually the first to become reactive, and did so six to 10 weeks after inoculation. The VDRL and RPR tests became reactive either concurrently or within two weeks of FTA-ABS conversion. Antibodies in the

TABLE III Response of rabbits to inoculation with popliteal lymph nodes from rabbits with naturally acquired infection with *Treponema paraluiscuniculi*

No of rabbit	Weeks followed	Titre of peak serological response in:			Clinical manifestations:				SI of lymphocytes from spleen
		VDRL	RPR	FTA-ABS	Lesions	DF	FADF	Orchitis	
1R	16	—	—	—	—	—	—	—	1.3
2R	15	—	—	—	—	—	—	—	1.1
3R	14	—	—	—	—	—	—	—	1.3
4R	15	—	—	—	—	—	—	—	1.4
5R	15	—	—	—	—	—	—	—	1.6
6R	26	1/16	1/16	1/512	Prepuce	+	+	+	1.2
7R-1	15	—†	—	—	—	—	—	—	2.3*
8R-1	22	1/16†	1/32	1/1024	Prepuce, face	+	+	+	1.0
7R-2	16	—†	—	—	—	—	—	—	1.0
8R-2	16	1/8	1/4	1/1024	Anus	+	+	—	1.3

VDRL = Venereal Disease Research Laboratory test; RPR = rapid plasma reagin test; FTA-ABS = fluorescent treponemal antibody absorbed test.

DF = dark field microscopy; FADF = fluorescent antibody dark field microscopy of exudate from lesions.

SI = Stimulation index (for explanation see table I); \* =  $p < 0.05$ .

† VDRL test reactive before inoculation.

— = Negative test result or absence of findings.

VDRL and RPR tests usually rose to titres of 1/8 to 1/16, with the titre in the VDRL test being slightly higher than in the RPR test. Antibody titres in the FTA-ABS test were appreciably higher, rising to 1/512 to 1/1024. Clinical disease was not apparent until 14 to 15 weeks after inoculation. Cutaneous lesions were erythematous, raised, or sessile, and their distribution was diffuse or focal. Lesions usually progressed to weeping and formation of crusts, then dried and became scaly before resolving. In two rabbits, clinical signs persisted for about seven weeks, whereas the third rabbit was killed two weeks

after onset of lesions. The relation of the serological response and clinical course of infection is depicted in the figure.

The inoculated left testicle was removed from rabbit 6R four weeks after the onset of genital lesions and from rabbit 8R-1 at the onset of lesions. Histopathological examination of the testicles showed pyogranulomatous orchitis with multifocal interstitial lymphoid infiltration. These foci often contained small granulomas with varying numbers of lymphocytes and neutrophils. Spirochaetes were observed in the centre of granulomas by the Warthin-Starry staining method. Rabbit 6R also showed a noticeable reduction in the numbers of spermatogonia and spermatids in the seminiferous tubules. The sham inoculated testicles were normal. Testicles removed from rabbit 8R-2 two weeks after the onset of genital lesions were also unremarkable.

Blood lymphocytes were assayed three weeks after serological evidence of infection (two weeks before the onset of lesions) in 8R-1 and nine weeks after serologic evidence of infection (two weeks after the onset of lesions) in 6R. The stimulation indexes were 1.0 and 1.6, respectively; the difference in uptake of  $^{125}$ IUDR between unstimulated lymphocytes and those exposed to treponemal antigen was not significant. Lymphocytes from the left popliteal lymph node were assayed five weeks after serological evidence of infection (at the onset of lesions) in 8R-1, 11 weeks after serological evidence of infection (four weeks after the onset of lesions) in 6R, and 12 weeks after serological evidence of infection (two weeks after the onset of lesions) in 8R-2. In all rabbits the stimulation index was 1.0. Transudate expressed from the lymph nodes was negative for treponemes by dark field microscopy, but positive by fluorescent antibody dark field microscopy in rabbits 6R and 8R-1. The left popliteal nodes were also examined for treponemes by fluorescent antibody staining, and low numbers of treponemes were demonstrable in rabbit 8R-1 but not in rabbits 6R or 8R-2. The inoculated testicles, removed at the same time as the lymph nodes from rabbits 6R and 8R-1, contained treponemes on dark field and fluorescent antibody dark field microscope examination of expressed fluid.

Recipients were killed at the end of the observation period, and splenic lymphocytes were assayed for immune responsiveness to *T. pallidum* antigen (table III). The uptake of  $^{125}$ IUDR by lymphocytes exposed to *T. pallidum* antigen was not significantly greater than for controls, with stimulation indexes ranging from 1.0 to 1.6 except in rabbit 7R-1. This rabbit had a significant lymphocyte response but was negative by all criteria of infection. Assessment of lymphocytes from the blood and popliteal nodes of

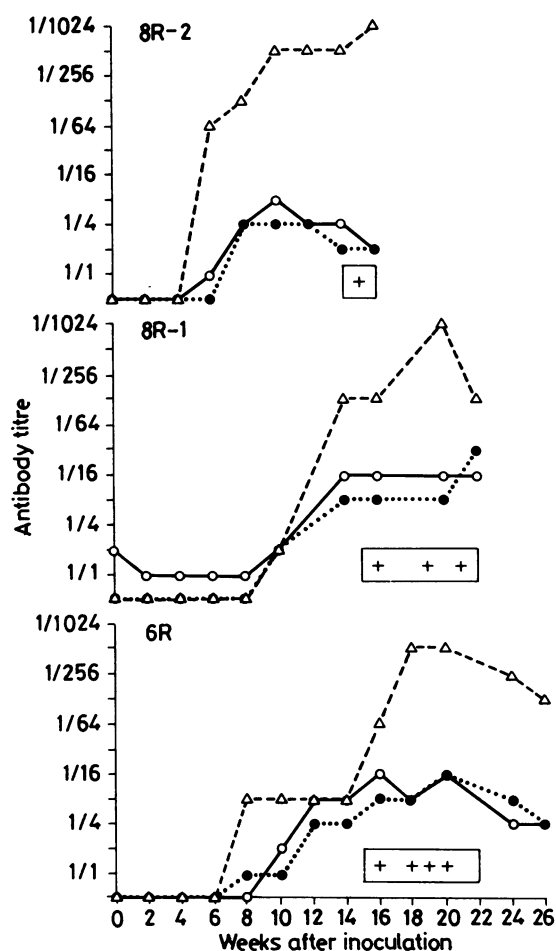


FIGURE Course of serological responses (○ = Venereal Disease Research Laboratory (VDRL), ● = rapid plasma reagin (RPR), △ = fluorescent treponemal antibody absorbed (FTA-ABS) tests in rabbits infected with *Treponema paraluis-cuniculi* by inoculation with popliteal lymph nodes from naturally infected rabbits. Box shows onset and duration of clinical signs of venereal spirochaetosis, with + indicating positive results on dark field and fluorescent antibody dark field microscopy.

experimentally infected rabbits showed no significant increase in  $^{125}\text{IUdR}$  uptake by lymphocytes exposed to *T pallidum* antigen.

In two of the successfully infected recipients, the inflammation scores in the left popliteal nodes were 9 and 11. Both popliteal nodes were obtained from the third successfully infected recipient, and the inflammation scores were 17 for the left node (inoculated side) and 4 for the right node (control side).

## Discussion

We evaluated the persistence of infection with *T paraluiscuniculi* in 11 New Zealand white rabbits that had clinical or serological evidence of venereal spirochaetosis. One rabbit had active lesions, another had been successfully treated for venereal spirochaetosis, and the remainder were asymptomatic. Eight of the 11 rabbits showed cellular immunity to *T pallidum* antigens in lymphocyte transformation tests. No evidence of *T paraluiscuniculi* could be shown in popliteal lymph nodes by fluorescent antibody staining of tissue sections or by dark field and fluorescent antibody dark field examination of lymph. Infectivity tests were performed by transferring extracts of lymph nodes to rabbits that were free of infection with *T paraluiscuniculi* on clinical examination and serological tests.<sup>15</sup>

Passive transfer of infection with *T paraluiscuniculi* was successful in three recipients. Popliteal lymph nodes obtained from rabbit 8D during both the acute and chronic stages of infection, and from rabbit 6D during the chronic stage of infection, successfully transmitted infection. Hence the only acutely infected rabbit and two of eight (25%) rabbits in the chronic phase successfully transmitted infection. The success of transferring infection with *T paraluiscuniculi* may depend on the cellular immune response in the donor. Of four rabbits in which the stimulation index of the transferred popliteal node (or the spleen, if the node was not sampled) was less than 2.5, two successfully transmitted infection. However, four rabbits with a stimulation index of more than 2.5 did not transmit infection. Successful infection of recipients was assessed by showing typical spiral organisms in dark field or fluorescent antibody dark field preparations of lesion scrapings, fluorescent antibody and silver staining of tissue sections, and seroconversion in the VDRL, RPR, and FTA-ABS tests.

Although earlier studies indicated a lack of seroreactivity in rabbits with venereal spirochaetosis,<sup>16 17</sup> more recent studies showed the development of reagin and treponemal antibodies during the course

of infection.<sup>18-20</sup> In the study reported here all donor rabbits were seroreactive and all experimentally infected recipients developed rising antibody titres in the VDRL, RPR, and FTA-ABS tests. A similar pattern of antibody formation occurred after experimentally infecting rabbits with *T pallidum*.<sup>2 10 21 22</sup>

Various workers have reported that the VDRL test has poor specificity in detecting treponemal infection in rabbits; positive reactions have been found in 10% to 30% of normal rabbits without apparent exposure to *T paraluiscuniculi*.<sup>1-5</sup> With few exceptions, serum from these rabbits was non-reactive in the FTA-ABS, TPI, or microhaemagglutination tests. Preinoculation serum samples from three of the 10 recipients in our study reacted in VDRL tests at dilutions of 1/1 to 1/4, but not in the RPR and FTA-ABS tests. This reactivity did not prevent transfer of *T paraluiscuniculi* infection to recipient 8R-1. Rabbits with histories of reactivity in the VDRL test only were as likely as non-reactive controls to develop dark field positive lesions after inoculation with *T pallidum*.<sup>2 4 23</sup> The lack of reactivity of the RPR test with preinoculation sera suggests that it is more specific than the VDRL test. Comparison with the FTA-ABS test showed that the RPR test was 100% and the VDRL test 87% specific in detecting *T paraluiscuniculi* infection.<sup>24</sup>

Persistence of *T paraluiscuniculi* has not been shown previously in naturally infected rabbits. In this study *T paraluiscuniculi* was seen in popliteal nodes when clinical signs of venereal spirochaetosis were evident, and the micro-organism persisted in these nodes for as long as 23 weeks. In a similar study, a recipient rabbit remained clinically well for three months after the transfer of a popliteal node from a naturally infected rabbit five months after clinical signs of venereal spirochaetosis had subsided.<sup>6</sup> In another study, infectivity tests were performed using popliteal nodes from nine rabbits that were VDRL reactive at dilutions of 1/1 to 1/16 for six to 18 months, but had no clinical signs of venereal spirochaetosis; four of the rabbits were also reactive in the FTA-ABS test.<sup>4</sup> Recipients did not show any clinical or serological evidence of venereal spirochaetosis during six to 12 months' observation. Neither did recipients of popliteal nodes from four non-reactive control rabbits. Popliteal nodes from five of eight rabbits with naturally acquired *T paraluiscuniculi* infection have been reported to produce dark field positive lesions in recipients.<sup>7</sup> No or minimum serological responses occurred in recipients, however, and pathological studies of nodes from selected recipients were negative for treponemes. Recipients of popliteal nodes from seven normal rabbits showed no clinical or serological evidence of venereal spirochaetosis.

Successful transfer of infection with *T paraluis-cuniculi* by popliteal nodes has been reported in experimentally induced infections in rabbits. Popliteal nodes from five of six rabbits in both the acute and chronic stages of infection after inoculation successfully transmitted infection to normal rabbits by intratesticular inoculation.<sup>6</sup> A review of studies by French workers showed that lymph nodes from five experimentally inoculated but clinically healthy rabbits successfully transmitted infection to normal rabbits.<sup>25</sup> Although the duration of infection was not always given, infectivity studies using experimentally infected rabbits appeared to be performed closer in time to clinical signs of venereal spirochaetosis than when using naturally infected rabbits. Duration of infection may therefore have influenced its transfer.

Although the total number of rabbits evaluated in various infectivity studies was small, persistence of *T paraluis-cuniculi* in the popliteal lymph nodes of naturally infected rabbits was not common. Successful transfer of infection may be related to the immune state. Most rabbits with natural *T paraluis-cuniculi* infections in this study had enhanced cellular immunity to treponemal antigen, but successful transfer of infection was apparently related to a poor cellular immune response. The greater success reported with popliteal nodes from experimentally infected rabbits may be related to the lack of cellular immune response in these rabbits, as experimentally infected rabbits in this study lacked a specific cellular immune response during the course of active disease. As this and other studies<sup>18-19</sup> have shown, intratesticular inoculation of rabbits with *T paraluis-cuniculi* results in the development of serum antibodies followed by the occurrence of cutaneous lesions several weeks later. Serum antibodies may block the cellular immune response and permit persistence of *T paraluis-cuniculi* in tissues as, in natural infection or intradermal inoculation of *T paraluis-cuniculi*, the onset of cutaneous lesions precedes the development of serum antibodies.<sup>20-26</sup> Comparison of intravenous and intradermal infection of rabbits with *T pallidum* suggests that factors in autologous serum may influence in vitro lymphocyte reactivity.<sup>27</sup>

Infection with *T paraluis-cuniculi* can persist in a latent state after both naturally and experimentally acquired infection. Reagin and specific treponemal antibodies develop after infection, with peak titres corresponding to the period of active clinical disease. After remission of genital lesions seroreactivity remains, and *T paraluis-cuniculi* may be shown in naturally infected rabbits by the transfer of lymph nodes to susceptible rabbits and, in addition, in experimentally infected rabbits by dark field and fluorescent antibody dark field microscopy of nodal

lymph or by fluorescent antibody and silver staining of lymph nodes. Most naturally infected rabbits develop cellular immunity to treponemal antigens, whereas rabbits infected experimentally by intratesticular inoculation fail to develop a cellular immune response to treponemal antigens during primary infection. Persistence of treponemes in a small number of naturally infected rabbits and in experimentally infected rabbits may be related to the absence of a specific cellular immune response.

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